



PATENT

Attorney Docket No. Lee&Li 103

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:)
En-Chung Lin)
Serial No.: 09/995,026)
Filed: November 27, 2001)
Examiner: Juliet Caroline Switzer)
Art Unit: 1634)
For: DETECTION OF GROWTH)
PERFORMANCE OF PIGS)

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DECLARATION OF DR. EN-CHUNG LIN

I, En-Chung Lin, hereby state as follows:

1. I, En-Chung Lin, am one of the co-inventors of U.S. Patent Application Serial No. 09/995,026, filed November 27, 2001 and entitled "Detection of Growth Performance of Pigs."

2. I am an Associate Research Fellow at the Animal Technology Institute in Taiwan. My resume is attached to this Declaration as Exhibit A.

3. Under my direction, experiments demonstrating the relationship and effects of each of the five (5) polymorphic

sites of the 5' flanking region of HSP70.2 gene on the traits of growth/backfat thickness in pigs were conducted. The experimental protocol is set forth below and the results are set forth in the attached Table (Exhibit B).

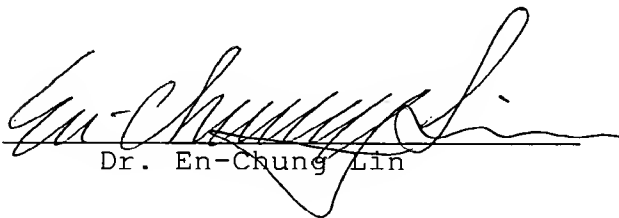
4. The blood samples were collected from 500 Duroc testing boars sent by 11 different purebred herds in Taiwan. Those testing boars were sent to the testing station at Animal Technology Institute Taiwan in Chunan, Taiwan at 17~20 days of age. They were raised in the same facility, feeds and management till reaching 110kg of body weight. DNA samples were purified from the blood samples and stored in -20°C. We selected DNA samples from 205 out of the 500 testing boars for subsequent sequencing of the 5' flanking region of HSP70.2 gene by avoiding genetic relationships among such boars to make the samples as random as possible.

5. The data of the 5' flanking region of HSP70.2 gene sequences and backfat thickness performance were analyzed using SAS GLM procedure. There were two fixed effects of season and each of the five nucleotide polymorphic sites was involved in the model. As shown in the attached Table, the five nucleotide polymorphic sites were found to have significant effects ($p < 0.1$ or 0.05) on backfat thickness. The mutation sites of P44 and P232 have weaker effects ($p < 0.10$) on backfat thickness, while the sites of P250, P345 and P393 have more significant effects

($p < 0.05$) on that performance trait. In addition, the frequencies of the mutation sites of P44 and P232 were found to be the same in the samples of the 205 boars with very limited genetic relationships. The genotype of AC on both sites has significantly thicker backfat than the other two genotypes of AA and CC. The frequencies of the mutation sites of P250, P345 and P393 were apparently different from each other. This means that the three mutation sites might not be transmitted together to next generations. Thus, their effects on backfat thickness are slightly different from each other but all of them show significant influence on backfat thickness.

6. But, the model has not considered all possible combinations of the five polymorphic sites simultaneously. Thus, we proposed a statistical model using haplotypes of those five nucleotide polymorphic sites in the 5' flanking region of HSP70.2 gene. The results showing the association between the haplotypes and backfat thickness are shown in the specification of patent application.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under the laws of the United States of America, and including 18 USC 1001, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.


Dr. En-Chung Lin

2003.1.6

Date

Appendix I

En-Chung Lin, Ph. D

Position:

Associate Research Fellow at Animal Technology Institute Taiwan

Education:

Ph.D. major in Animal Breeding/Quantitative Genetics, minor in Biostatistics, Dept. of Animal Science, Iowa State University (1997.5)

M.S. in Animal Breeding, Dept. of Animal Science, Iowa State University (1992.12)

B.S., Dept. of Animal Husbandry, National Chung-Hsing University (1985.6)

Experience:

Associate Research Fellow, Dept. of Applied Biology, Animal Technology Institute Taiwan (2001.9~now)

Associate Research Fellow, Dept. of Animal Resources, Pig Research Institute Taiwan (1997.4~2001.8)

Section Leader, Section of Informatics, Dept. of Plan and Service, Pig Research Institute Taiwan (1997.7~1999.8)

Research Assistant, Dept. of Animal Science / Computational Center, Iowa State University (1990.6 ~1997.4)

Substitute Teacher in Biology, Hon-Dou Middle School, Taipei City (1989.1~1989.6)

Research Assistant, Sections of General Surgery / Allergy, Immunology and Rheumatology, Taipei Veteran General Hospital (1988.1~1988.11)

Research Interest:

Bioinformatics / Computational Biology for Genomic study in livestock animals

Genetic Regulatory study and application of growth / heat tolerance / meat quality in livestock animals

Study and application for Segregated Early Weaning production system in Pigs

Establishing and related products by using HACCP pig production model

Genetic Evaluation for livestock animals

National Breeding and Performance Testing systems in Pigs

Biostatistics Methods:

Restricted maximum likelihood (REML) with Expectation Maximization (EM) or Derivative-Free (DF) algorithm for (co)variance component estimation;

Best linear unbiased estimation or prediction (BLUE, BLUP) using mixed linear model;

Box-Cox transformation for adjusting data sets to normality assumption;

Gibbs Sampling (using MCMC method) for providing posterior distribution of particular parameters;

Multivariate analysis for considering many traits at the same time;

Linear model for conducting genetic evaluation;

Experimental design for obtaining most inferences from experiment.

Research Projects:

PI: Primary Investigator; Co-PI: Cooperative Investigator; NSC: National Science Council; COA: Council of Agriculture

1. Establishing the bioinformatics center for swine research in tropical area (a project of bioinformatics for Functional genomics and Proteomics cooperated with other 4 projects) (PI, COA) 2002/1/1~2004/12/31
2. Studies on the porcine and chicken functional genome and the development of cDNA chips for reproduction trait — porcine EST construction (a sub-project of Bioinformatics) (Co-PI, NSC/COA) 2002/8/1~2004/12/31
3. The Genetic Evaluation and Competition Analysis Between Domestic and Imported Commercial Hog (PI, COA) 2002/1/1~2002/12/31
4. Developing inbred line in pigs as animal model for human Hypertrophy Cardiomyosin (PI, NSC) 1997/8/1~2000/7/31
5. Association between polymorphism the 5' flanking region of Heat Shock Protein 70.2 gene and growth/backfat performance (PI, NSC) 2000/8/1~2001/7/31
6. The genetic analysis and its relationship with economical traits of heat stress protein 70 (PI, NSC) 1999/8/1~2000/7/31
7. Verification of Multiple-trait Heterogeneous Mixed Linear Model (PI, NSC) 1998/8/1~1999/7/31
8. Genetic Evaluation of Breeding Animals and their progeny in Growth and

Reproductive Performance (PI, COA) 1998/7/1~2001/12/31

9. Establishment of Pig Production Model using HACCP system (PI, COA) 1999/12/1~2000/12/31
10. The Comparison of Semen Quality and Conception Rate between Wet Pad / Forced Ventilation and Open boar houses (Co-PI, COA) 1998/7/1~1999/6/30
11. Efficiency Evaluation for Multiple-site Segregated Early Weaning System (Co-PI, COA) 1998/7/1~1999/6/30
12. Establishment and Integration of Database for Pig Industry (PI, COA) 1997/7/1~1999/6/30
13. A Computer System for Animal Epidemiology (PI, COA) 1997/7/1~1998/6/30

Publications:

(omitted)

Table The effects of each of the five polymorphism sites in the 5' flanking region of HSP70.2 gene on the trait of backfat thickness

Mutation site		BF
P44	AA (4)	1.40±0.06 ^{de}
	AC (32)	1.46±0.03 ^d
	CC (169)	1.39±0.02 ^e
P232	AA (4)	1.40±0.06 ^{de}
	AC (32)	1.46±0.03 ^d
	CC (169)	1.39±0.02 ^e
P250*	AA (123)	1.41±0.02 ^a
	AO (72)	1.40±0.02 ^a
	OO (10)	1.31±0.04 ^b
P345	CC (7)	1.43±0.05 ^{ab}
	TC (33)	1.45±0.03 ^a
	TT (165)	1.39±0.02 ^b
P393	CC (111)	1.42±0.02 ^a
	TC (81)	1.37±0.02 ^b
	TT (13)	1.37±0.04 ^{ab}

BF: backfat thickness (cm) adjusted to 110kg of body weight

a, b: least square means with different superscripts differ significantly among genotypes (P<0.05)

d, e: least square means with different superscripts differ significantly among genotypes (P<0.10)

*: The symbol "O" in the genotypes of AO and OO means deletion of a base at the P250 site.